

<p>(51) International Patent Classification 7 : B01J 2/04, A61K 9/16</p>	<p>A1</p>	<p>(11) International Publication Number: WO 00/56439</p> <p>(43) International Publication Date: 28 September 2000 (28.09.00)</p>
<p>(21) International Application Number: PCT/SE00/00566</p> <p>(22) International Filing Date: 22 March 2000 (22.03.00)</p> <p>(30) Priority Data: 9901059-7 22 March 1999 (22.03.99) SE</p> <p>(71)(72) Applicants and Inventors: CARLFORS, Johan [SE/SE]; Prästgårdsgatan 13 C, S-752 30 Uppsala (SE). GHADERI, Raouf [SE/SE]; Djäkneg. 87-230, S-754 25 Uppsala (SE).</p> <p>(74) Agents: BERG, S., A. et al.; Albihns Patentbyrå Stockholm AB, P.O. Box 5581, S-114 85 Stockholm (SE).</p>		<p>(81) Designated States: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).</p> <p>Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p>

The diagram illustrates a high-pressure processing system. A central rectangular chamber, labeled 'High Pressure Vessel' with an arrow pointing to it, contains a smaller rectangular 'OVEN' at the bottom right. The vessel is labeled with the number '2'. The oven is labeled with the number '4'. A fluid circuit is shown with various components and numbered labels:

- Fluid Sources:** Two vertical cylindrical tanks are on the left, labeled '20' (top) and '8' (bottom).
- Flow Control:** A valve, represented by a circle with an 'X', is labeled '22' and is connected to tank '20'.
- Pressure Measurement:** A pressure gauge, represented by a circle with concentric arcs, is labeled '24' and is connected to the line from tank '20'.
- Heating/Processing Elements:** Two vertical rectangular blocks with a stippled texture are labeled 'SKV-D' (top) and 'SKV-E' (bottom).
- Flow Direction:** Arrows indicate the flow path. An arrow labeled '6' points from SKV-D to SKV-E. An arrow labeled '8' points from SKV-E into the high-pressure vessel.
- Internal Components:** Inside the high-pressure vessel, there is a 'Nozzle' at the top right and a 'Filter' below it. Two small rectangular components, both labeled '28', are also shown inside the vessel.
- Pressure Measurement:** A pressure gauge labeled '12' is connected to the bottom of tank '8'.
- Exit and Heating:** A line exits the bottom of the high-pressure vessel, labeled '30'. This line passes through a horizontal rectangular block with a lens-like shape, labeled '32'. After this block, the line is labeled '4' and enters the 'OVEN'.
- External Pressure:** A pump, represented by a circle with a 'P', is labeled '2' and is connected to the top of the oven.

BEST AVAILABLE COPY

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LJ	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

Method for the preparation of particles

This invention relates to the preparation of particles and, more specifically, to a new method for the preparation and an apparatus therefor. In a further aspect, the invention also relates to particles prepared by the method and their use in different areas.

Background of the invention

Traditionally, particles have been prepared by milling of solids or by crystallisation from solutions.

In later years, however, new methods for the preparation of particles have been developed, which methods comprise the precipitation of particles from solutions in media consisting of supercritical fluids. The advantages of these newer methods for the preparation of particles are several.

- Because the solution properties of supercritical fluids to a great extent are dependent on pressure and temperature, these properties can easily be controlled and varied. This means possibilities to precipitate/crystallise a substance with different properties as to size, form, and morphology. Furthermore, the supercritical fluid may specifically extract impurities in the substance.
- The method is a continuous process with possibilities to recover solvents and to recycle the supercritical medium. Consequently, the process is well suited for industrial production.
- As media for supercritical fluids, environmentally acceptable gases can be used e g carbon dioxide. Other suitable gases are nitric oxide, sulphur hexafluoride, ethylene, ethane etc.

In methods for the preparation of this kind, a solution or dispersion is introduced with the actual substance(s), denoted "substance liquid" below, into the supercritical medium, wherein the substance(s) is/are precipitated, on one hand, as a consequence of decreased solubility properties and, on the other hand, in consequence of extraction of the solvent of the substance liquid by the supercritical fluid, a so called "gas anti-solvent technique". At present, the method exists in different technical designs. One example is the introduction of the substance liquid into the supercritical fluid via dispersion, atomisation of the solution in a spray nozzle, whereupon the dissolved substance(s) is/are precipitated in the form of particles upon contact with the supercritical fluid.

The dispersion via the spray nozzle is achieved in different ways. One way is to apply a pressure gradient over the nozzle, the opening diameter of which is small (J. Bleich et al, International Journal of Pharmaceutics, 97 (1993) 111-117; Yeo et al, Biotechnology and Bioengineering, Vol. 41, 341-346 (1993). Another way is to atomise the drops with ultrasound. In these methods, the substance liquid is either sprayed into a container already filled with SCF or in a continuous process, wherein SCF is continuously introduced into the container via a separate channel. The continuous process has a higher production yield.

Another continuous process is to use a coaxial nozzle, wherein the substance liquid is introduced into a channel, whereas the supercritical fluid is introduced into the other channel and, thereby, facilitates the dispersion of the substance liquid. In this method (SEDS method), the particles are directly formed during the dispersion, because the supercritical fluid is introduced simultaneously with the substance liquid. The supercritical fluid (US-5,851,453, Mazen et al) achieves both dispersion and extraction of solvent from the substance liquid. This method means better possibilities to control particle properties such as particle size and form, as the dispersion is dependent on the flow rate of the two liquids, and the design of the spray nozzle itself. A problem with this technical design of the spray nozzle, however, is that the particle formation process is already initiated in the nozzle

itself, as the substance liquid is brought in contact with the supercritical fluid. This means, for some substances, that the precipitation thereof begins before the dispersion of the liquid. In some cases, no particles at all are thereby obtained and, in other cases, particles are obtained but with relatively large diameters. Another problem with this process is that the premature precipitation of substance sometimes is localised to the inner walls of the spray nozzle. This leads to a time dependent change of the inner diameter of the nozzle during the process. As a result, the particle size distribution of the final product thereby will be broadened leading to a less well-defined product. In some cases, the precipitation in the spray nozzle can lead to a total blockage thereof.

According to another patent (US-5,874,029, Subramaniam et al 1999), an attempt has been made in order to diminish some of the disadvantages of the SEDS method mentioned. The inventors thereby used compressed liquid instead of supercritical fluids, in order to atomise the substance liquid before the drops formed were exposed to the solvent extracting effect from the anti-solvent, in the form of a supercritical fluid. These liquids are preferably compressed CO₂ or light gases mixed with CO₂. However, as also is mentioned by the inventors, the anti-solvent effect diminishes in the supercritical fluid as it is mixed with these compressed gases. There is even a possibility of a non-supercritical state occurring in the reaction vessel because of this leading to a reduced capacity in producing particles from the substance liquid. Another disadvantage of the preferred use of CO₂ mentioned as the compressed liquid is that its own extraction capacity may lead to precipitation of the particle forming substance already in the nozzle leading to a less well defined product as mentioned before.

WO 9836825 A1 (Bradford Particle Design Ltd.) relates to a method and apparatus for the formation of particles using a solution or suspension of a substance in a liquid and a first and a second supercritical fluid, respectively. According to this document, both supercritical fluids are introduced simultaneously in the same fluid inlet assembly. However, many types of particles e.g. amorphous particles are

difficult to prepare by such a process. A solution to this problem is to add the first supercritical fluid in order to disperse a substance liquid and then, in a separate step, to add the second supercritical fluid order to extract the liquid carrier medium. The invention constitutes an improvement in preparing many kinds of particles,
5 especially particles of amorphous substances such as the particles composed of amorphous polymers prepared by the invention.

Methods using two separately added supercritical fluids are previously known e.g. as described in JP 7008202 A (Lion Corporation). However, this method does not
10 relate to a dispersion and extraction step for the preparation of particles but to a solvent extraction method in order to produce a spice extract.

Summary of the invention

15 Accordingly, there is a need of improved methods for the preparation of microparticles and nanoparticles.

In accordance with this invention, we have solved the problems associated with present methods by a new principle for the preparation of particles in supercritical
20 fluids. According to this new principle, the substance liquid is sprayed through a suitably designed nozzle e g a coaxial spray nozzle together with a supercritical fluid, SCF-D (D stands for dispersion) characterised in that it has a very low extraction capacity with regard to the solvent for the substance solution. Ideally, this SCF-D should lack interactions with the solution. This SCF-D should be chosen in
25 order to have minimal interactions with the substance liquid. SCF-D is only added in the purpose of increasing the dispersion of the substance liquid that later on, via the spray nozzle is sprayed into the reaction container in the form of drops. Not until then, the particle formation process starts through extraction of the solvent and a reduced solubility of the particle forming substance. The particle forming process
30 takes place through the addition of another supercritical fluid, SCF-E (E stands for extraction) via a separate addition channel to the reaction container. SCF-E is

characterised in that it has a high extraction capacity with regard to the solvent or dispersing agent of the substance liquid.

5 One object of the invention is a method for the preparation of particles comprising the following steps:

- (i) the dispersion, with a first supercritical fluid (SCF-D) of a substance liquid comprising a mixture of, at least, one substance of which particles are to be prepared, and a liquid carrier medium; and
- 10 (ii) the subsequent extraction, with another supercritical fluid (SCF-E), after the dispersion of the substance liquid, of the liquid carrier medium.

15 In particular, the dispersion takes place in a nozzle spraying the dispersed mixture into a reaction vessel, the pressure and temperature of which is controlled leading to a supercritical state in the reaction vessel.

It is preferred that the extraction takes place inside the reaction vessel.

20 The supercritical medium with extracted solvent is removed continuously from the reaction vessel via an outlet system being particularly designed according to the invention in order to promote and support the extraction capacity in the process. The process is continuous and, accordingly, well suited for production of large batches.

25 According to the invention, the two supercritical fluids have to be chosen starting from the criterion that the mixture of the liquids formed in the reaction vessel must exist in a supercritical state in order to ensure the extraction capacity of the supercritical fluids. This can be achieved with a SCF-D having lower critical parameters than those of SCF-E. One example is to use N₂ as SCF-D and CO₂ as
30 SCF-E.

The use of a supercritical fluid, SCF-D, according to the invention instead of compressed gases in order to disperse the liquid is advantageous, since it results in a well preserved extraction capacity of the supercritical medium, together with the prevention of a too early precipitation of substance in the nozzle.

5

Those substances that can be used in order to prepare particles according to the invention can be of very different kinds. One example is polymers, amorphous, semi-crystalline or crystalline, organic substances, and inorganic substances such as metals, oxides, ceramics, salts, or mixtures of these. As a carrier medium for those substances solvents and dispersion media are suitable, or mixtures of two or more solvents and dispersing agents, suitably with a low toxicity. Some non-limiting examples are water, lower ketones such as acetone, lower hydrocarbons such as hexane, lower alcohols such as methanol, ethanol, isopropanol, ethyl acetate, or mixtures thereof. Even chlorinated hydrocarbons can be used but they are not preferred because of their toxicity.

10

15

It is preferred that the substance(s) is/are synthetical or natural polymers, solid organic substances, solid inorganic substances, salts, biomolecules such as proteins e g enzymes. The substance(s) is/are preferably bioactive for therapeutic use.

20

Another object of the invention is an apparatus for the preparation of particles, comprising:

a particle formation reactor 2, the pressure and temperature of which are controllable;

25

a means 18, 2 in order to deliver to the apparatus a substance liquid comprising
a) at least one substance of which the particles are to be prepared, and
b) a liquid carrier medium for the substance;

30

a dispersing means 17 for the dispersion of the substance liquid and for the feeding of the dispersed substance liquid to the particle formation reactor 2;

5 a first feeding means 22, 17 for the feeding of a first supercritical fluid (SCF-D), with a minimal extraction capacity for the liquid carrier medium, to the dispersing means; and

10 a second, separately located, feeding means 12, 6 for the delivery into the particle formation reactor 2 of a second supercritical fluid (SCF-E) with a maximal extraction capacity for the liquid carrier medium, wherein the second supercritical liquid is introduced in the particle formation reactor 2 through a separate introduction channel.

15 Preferably, in the apparatus according to the present invention, the dispersing means comprises a nozzle with separate channels for the substance liquid and the first supercritical fluid (SCF-D), respectively. It is preferred that the delivery channels are designed as a coaxial spray nozzle. It is also preferred that the apparatus comprises a first outlet channel placed in the upper part of the reaction vessel and a second outlet channel placed in the lower part of the reaction vessel, in particular
20 wherein the outlet channels are provided with filters.

Furthermore, it is preferred that the reaction vessel is a high pressure chamber and is provided with a thermostat and a pressure regulator.

25 We have prepared particles of different substances, both by the SEDS method, and also according to present invention. Particles prepared by the SEDS method showed the disadvantages described above, whereas particles prepared according to the invention made the production of particles possible with smaller sizes and with narrower size distributions.

30

Furthermore, the product yield according to the present invention was higher compared to the SEDS method. The problems mentioned with variations of the inner diameter of the nozzle during the process could totally be eliminated using the invention.

5

Another object of the invention is particles prepared by the method as described above, comprising one or more pure or mixed substances. Preferably, the particles comprise one or more substances incorporated in one or several other substances. In particular, the particles comprise substances having activity as pharmaceuticals.

10

The invention will now be closer illustrated with a detailed description of examples of the preparation of particles of different materials and of a preferred embodiment of the apparatus according to the invention.

15

In the drawings:

Fig. 1 is a survey view of an apparatus according to the invention;

20

Fig. 2 is a SEM photograph of DL-PLG particles prepared by the method according to the invention;

Fig. 3 is a SEM photograph of DL-PLA particles prepared by the method according to the invention;

25

Fig. 4 is a SEM photograph of L-PLA particles prepared by the method according to the invention;

Fig. 5 is a SEM photograph of DL-PLG particles with incorporated hydrocortisone, prepared by the method according to the invention;

30

Fig. 6 is a SEM photograph of lysozyme particles prepared by the method according to the invention;

5 Fig. 7 is a SEM photograph of hydrocortisone particles prepared by the method according to the invention;

Fig. 8 is a SEM photograph of DL-PLG particles with incorporated urease, prepared by the method according to the invention.

10 Detailed description of preferred embodiments

The main principle for the preparation of particles from chemical substances according to the invention comprises the dispersion of a substance liquid in a reaction vessel in the presence of a supercritical fluid. The mechanism for the
15 formation of particles is that the substance liquid is dispersed in the form of droplets being induced by the simultaneous introduction of SCF-D into the vessel e.g. through a coaxial spray nozzle. The nozzle design according to the invention can vary but includes all nozzles designed in a manner that SCF-D is introduced into the reaction vessel together with the substance liquid which results in the SCF-D flow
20 improving the substance liquid dispersion. The substance liquid flow rate and SCF-D flow rate and their mutual relationships are important for the particle formation and have to be well controlled. The inner diameters of the openings for the channels, through which SCF-D and the substance liquid are introduced into the reaction vessel, have a great importance for the dispersion and, thereby, the particle
25 formation process. The reaction vessel consists of a thermostat-controllable high-pressure chamber. When the droplets are sprayed into the reaction vessel, the dissolved or the dispersed substance is converted to a solid phase, particles are formed by precipitation or by crystallisation. The particle formation takes place by extraction of the drop solvent or drop dispersing agent whereby the solvent or
30 dispersing agent is dissolved in the SCF-E in the reaction vessel. A contributing cause to the particle formation is also the reduced solubility of the substance(s) as a

result, on one hand, of the extraction of the solvent or the dispersing agent and, on the other hand, of the fact that the solubility of the substance(s) in the supercritical medium is much lower than in the original solvent (anti-solvent effect). SCF-E is introduced into the reaction vessel through a separate introduction channel. The SCF-E flow should be adapted with regard to the volume of the reaction chamber and with regard to the substance liquid flow with the intention to achieve an adequate high capacity for the extraction process. The particles formed are accumulated in the reaction vessel during the continuous process, whereas the supercritical medium containing extracted solvent or dispersing agent is removed through outlet channels provided with filters. These are designed in order to ensure that enough SCF-E is being maintained in the reaction vessel compared to SCF-D which makes the extraction capacity during the process as efficient as possible. Thereby, the process becomes continuous. After a finished process, the reaction vessel is opened and the product is collected.

Thus, the invention comprises a method according to a new principle for the preparation of particles and an apparatus for the preparation of particles. One embodiment of an apparatus is shown in Fig. 1. In this context it should be noted that this is only one of several possible designs of the principle comprised by the invention.

Accordingly, an apparatus according to the invention comprises a reactor or a reaction vessel 2, wherein the particle formation takes place. The reactor is accommodated in an oven 4 with a temperature control unit in order to make temperature control and regulation possible inside the reactor. Furthermore, there is a means 6 for the delivery of a first supercritical fluid (SCF-E) to the inside of the reactor. The supercritical fluid is obtained from a suitable gas being stored in a gas bottle 8 placed outside the surrounding oven. The gas from the bottle is cooled in a cooler 10 and is fed to the reactor by means of a high-pressure pump 12 via a heat exchanger 14 and a pulse damper 16. The pump 12 and the heat exchanger 14 are run in order to transfer the gas to a supercritical state. When delivered to the reactor

2, the gas exists in a supercritical state. The oven 4 is then run and regulated in order to maintain this supercritical state during the operation of the apparatus.

5 The apparatus is also provided with a coaxial dispersing nozzle 17. Such nozzles are well known per se and mainly comprise an inner and an outer channel surrounding the inner channel. A substance liquid is fed through the inner channel from a storage
bunker 18 situated outside the apparatus. In the outer channel, a supercritical fluid (SCF-D) is fed. This supercritical fluid is kept in a gas bottle 20 placed outside the
10 apparatus. It is possible to feed the substance liquid in the outer channel and to deliver the supercritical fluid through the inner channel but this is not as
advantageous, because the dispersion becomes better the smaller diameter of the channel for the substance liquid. There could also be more than one channel for the
substance liquid if one desires to deliver different liquids containing different
substances.

15

The apparatus is run in the following manner:

CO₂ (SCF-E) is fed from the gas bottle 8 to the refrigerating machine 10 in order to
be cooled to a liquid state. After that, it is fed to the high-pressure pump 12. It is
20 pumped further with a constant flow from the high-pressure pump to the reaction
vessel 2 via the heat exchanger 14 and the pulse damper 16. SCF-D is fed from the
vessel 20 via a thin needle valve that gives a constant SCF-D flow to the heat
exchanger 24 and is, after that, fed to the coaxial nozzle 17, optionally via a pulse
damper 19. The substance liquid is fed from the vessel 18 via the high-pressure
25 pump 21 through the inner channel of the coaxial nozzle 17. SCF-D disperses the
substance liquid by pressing it through the nozzle opening inside the high-pressure
vessel 2. The supercritical medium leaves the high-pressure vessel via two openings
26, 28 provided with filters placed in the upper and the lower part of the high-
pressure vessel 2, respectively. The orifices then converge to the outlet channel 30
30 and the medium is fed to the back pressure regulator 32.

- The coaxial nozzle opening may vary from 0.02 to 2 mm.
- The pressure difference between the SCF-D vessel 20 and the pressure vessel 2 is at least 5 bars and preferably higher.
- The SCF-D flow is sufficiently high in order to achieve a sufficient dispersion of the substance liquid in the chosen nozzle, preferably at least 2 standard litres per minute (in a supercritical state).
- The SCF-E (CO₂) flow in a liquid state should be high enough in order to achieve a sufficient extraction capacity in the chosen pressure vessel, preferably at least 10 ml per minute.
- The liquid flow rate of the substance liquid should be sufficiently low in order to ensure a sufficient high solvent extraction to produce well-defined particles. This flow rate is dependent on the properties of the dissolved substance, the solvent, the extraction properties of the supercritical medium and the volume of the reaction vessel.
- The mutual relationships between the three liquid flows, the volume of the reaction vessel, and the opening diameters of the inlet orifices are optimised to give particles with desired properties.
- The reaction chamber is a thermostat-controllable high-pressure vessel, the volume of which is sufficiently big to give a sufficient extraction capacity. The temperature is controlled in order to give a suitable temperature above or below the critical point.
- The reaction vessel pressure may be kept constant and may be controlled with a back pressure regulator.

As used in this application:

- a) the term "supercritical fluid" (denoted SCF below) refers to the phase state (supercritical state) that exists when a liquid is subjected to pressures and temperatures above the critical point;
- b) the term "critical point" regarding the temperature i e critical temperature refers to that temperature above which a gas cannot be liquefied by pressure alone. The

pressure under which a substance may exist as a gas in equilibrium with the liquid at the critical temperature is the critical pressure. The critical temperatures and pressures of many compounds can e.g. be retrieved from "Handbook of Chemistry and Physics", 58th Ed., Crc Press, Inc.;

- 5 c) the term "extraction capacity" refers to the ability of an extraction medium to dissolve another substance or liquid;
- d) the term "lower critical parameters" refers to lower critical temperature and lower critical pressure;
- 10 e) the term "substance liquid" refers to a liquid comprising a solution or a dispersion containing one or more substances of which one desires to prepare particles dissolved or dispersed in a solvent or a dispersing agent, or in a mixture of two or more solvents or dispersing agents;
- f) the term "compressed gas" refers to a gas brought to a lower volume and higher pressure than it otherwise would have at the same temperature;
- 15 g) the term "SEM photograph" refers to a scanning electron microscope photograph;
- h) the term "s.d." refers to standard deviation;
- i) the prefix "L" of a compound (such as L-PLA) refers to a stereoisomer i.e. the levorotatory form of the compound in the respect of plane polarised light;
- 20 j) the prefix "DL" of a compound (such as DL-PLG and DL-PLA) refers to another stereoisomer i.e. the racemic form that is not optically active in the respect of plane polarised light;
- k) the term "w/v" refers to a weight percentage of a substance dissolved or dispersed in a liquid in terms of the volume thereof; and
- 25 l) the term "w/w" refers to a weight percentage of a substance dissolved or dispersed in a liquid in terms of the weight thereof; and
- m) the term "codisperse" refers to the simultaneous dispersion of two liquids.

30 The invention is now illuminated by means of the following Examples, which only illustrates the invention and do not limit the scope thereof.

EXAMPLES

Materials and methods

5 DL-PLG is poly-DL-lactic glycolic acid, DL-PLA is poly-DL-lactic acid, and L-PLA is poly-L-lactic acid. They are all biodegradable polyesters, DL-PLG and DL-PLA being copolymers and L-PLA being a homopolymer. DL-PLG, DL-PLA, and L-PLA were purchased from Birmingham Polymers Inc., USA. Lysozyme from chicken egg white, hydrocortisone, and urease were purchased from Sigma, USA.

10

Example 1

Particles were prepared from the polymer DL-PLG (50:50) that is amorphous. A 2.3% (w/v) solution of the polymer in a mixture of organic solvents (acetone: ethyl acetate: isopropanol, volume fraction 4:5.6:0.4) has been dispersed by means of N₂ as SCF-D. CO₂ was used as SCF-E. The polymer solution flow was 0.13 ml per minute and the SCF-D flow was 12 litres per minute (in a supercritical state). The SCL-E flow was 21 ml per minute (in a liquid state).

15

20 The chosen nozzle opening was 0.2 mm. The pressure was 130 and 160 bars, respectively, and the temperature was 38°C and was kept constant during the process.

The particles were discrete and had an almost spherical form (Figure 2). The particle size distributions were relatively narrow (Table I).

25

Example 2

Particles were prepared from the polymer DL-PLA that is semi-crystalline. A 2.3% (w/v) solution of the polymer in a mixture of organic solvents (acetone: ethyl acetate: hexane, volume fraction 1:7.8:1.2) has been dispersed by means of N₂ as

30

SCF-D. CO₂ was used as SCF-E. The polymer solution flow was 0.15 ml per minute and the SCF-D flow was 12 litres per minute (in a supercritical state). The SCF-E flow was 21 ml per minute (in a liquid state).

5 The chosen nozzle opening was 0.2 mm. The pressure was 130 and 160 bars, respectively, and the temperature was 35°C and was kept constant during the process.

10 The particles were discrete and had an almost spherical form (Figure 3). The mean particle size was approximately 10 µm that was independent of the pressure (Table I).

Example 3

15 Particles were prepared from the crystalline polymer L-PLA. A 2.3% (w/v) solution of the polymer in a mixture of organic solvents (methylene chloride: acetone: isopropanol, volume fraction 3.3:6.5:0.2) has been dispersed by means of N₂ as SCF-D. CO₂ was used as SCF-E. The polymer solution flow was 0.15 ml per minute and the SCF-D flow was 12 litres per minute (in a supercritical state). The
20 SCF-E flow was 21 ml per minute (in a liquid state).

The chosen nozzle opening was 0.2 mm. The pressure was 130 and 160 bars, respectively, and the temperature was 40°C and was kept constant during the process.

25 The particles were discrete and had an almost spherical form (Figure 4). The mean particle size was approximately 7 µm that was independent of the pressure (Table I).

30 Table I:

The particle size distribution from particles prepared by the method according to the invention

Diameter ^a (μm) of microparticles						
Pressure (bars)	130			160		
Polymer, T (°C)	10%	50%	90%	10%	50%	90%
DL-PLG (0,78) ^b , 38°C	2.8	5.6	10.7	3.1(0.1)	8(2)	38(30)
DL-PLA, 35°C	3.9	9.9	26.3	3.8(0.0)	10.2(0.4)	55(29)
L-PLA, 40°C	2.5(0.2)	7(2)	24(12)	2.4	7.9	37.7

5 ^aThe standard error within parentheses is based on measurements from three batches

^bInherent viscosity

Example 4

10 The invention has also been shown to be useful in incorporation of active substances (medical or chemical ones) in another substance. Examples from such an experiment are particles prepared from DL-PLG (50:50) with incorporated hydrocortisone. A 2.3% (w/v) polymer and 0.12 % (w/v) hydrocortisone solution in a mixture of organic solvents (acetone: ethyl acetate: isopropanol, volume fraction
15 4.15: 4.85: 1.0) has been dispersed by means of N₂ as SCF-D. CO₂ was used as SCF-E. The polymer solution flow was 0.15 ml per minute and the SCF-D flow was 12 litres per minute (in a supercritical state). The SCF-E flow was 21 ml per minute (in a liquid state). The chosen nozzle opening was 0.2 mm. The pressure was 135 bars and the temperature was 38°C and was kept constant during the process.

20

The particles were discrete and the mean particle size was approximately 9 μm (Figure 5). 51 % of the added amount of hydrocortisone was incorporated in the polymer particles (Table II).

Table II: volumetric particle size distribution and the relative amount of incorporated hydrocortisone (hc) and urease (u) in regard of the added amount of the two substances for DL-PLG particles.

Substance, Pressure (bars)/ Temperature (°C)	Diameter ^a (μm) of microparticles			% EE ^b
	10%	50%	90%	
hc, 135/38	3(0)	9(1)	30(8)	51(3)
u, 160/38	3(0)	6(1)	18(4)	46(3)

^aThe standard error within parentheses is based on measurements from three batches. ^bThe entrapment efficiency, EE, is the fraction of the substance which was incorporated of the added amount of substance to the process.

Example 5

Lysozyme microparticles were prepared by dispersion of lysozyme in a mixture of 0.001 M HCl and ethanol (volume fraction 9.1:0.9) in supercritical CO₂ as SCF-E by using N₂ as SCL-D. The lysozyme concentration was 14.6% (w/w). The protein solution flow rate was 0.02 ml per minute, with a N₂ and CO₂ flow rate of 8 standard litres per minute (in a supercritical state) and 21 ml per minute (in a liquid state), respectively. The process pressure was 155 bars and the process temperature was 40°C. The nozzle orifice was 0.2 mm.

The lysozyme particles obtained were discrete and had a volumetric particle size from 1 to 5 μm (see Fig. 6).

Example 6

Hydrocortisone was dissolved in a mixture of acetone and ethanol (7.5:2.5). The hydrocortisone concentration was 12.8% (w/w). The solution was dispersed in
5 supercritical CO₂ as SCF-E by using N₂ as SCF-D. The flow rate of the hydrocortisone solution, N₂, and CO₂ was 0.152 ml per minute, 12 standard litres per minute (in a supercritical state), and 21 ml per minute (in a liquid state), respectively. The process pressure was 130 bars and the process temperature was 40°C. The nozzle orifice was 0.2 mm.

10 The hydrocortisone particles obtained were a mixture of discrete irregular and needle-shaped particles with a particle size from 1 to 5 µm (see Fig. 7).

Example 7

15 The invention has also been shown to be useful in incorporation of active substances (medical or chemical ones) in another substance. Another example from such an experiment are particles prepared from DL-PLG (50:50) with incorporated urease. A 2.3% (w/v) polymer and a 0.12% (w/v) urease solution in a mixture of
20 organic solvents (acetone: ethyl acetate: isopropanol, volume fraction 4.15: 4.85: 1.0) and water respectively were codispersed by means of N₂ as SCF-D. CO₂ was used as SCF-E. The polymer solution flow was 0.15 ml per minute and the SCF-D flow was 12 litres per minute (in a supercritical state). The SCF-E flow was 21 ml per minute (in a liquid state). The chosen nozzle opening was 0.2 mm. The pressure
25 was 160 bars and the temperature was 38°C and was kept constant during the process.

The particles were discrete and spherical. The mean particle size was 6 µm (Figure 8). 46% of the added amount of urease was incorporated in the polymer particles
30 (Table II). Furthermore, 60% of the urease was released, in vitro, from the

microparticles during one month. The enzymatic activity of the released urease from the microparticles was 82 ± 4 (s.d.) %.

Claims

1. A method for the preparation of particles comprising the following steps:

- 5 (i) the dispersion, with a first supercritical fluid (SCF-D) of a substance liquid comprising a mixture of, at least, one substance of which particles are to be prepared, and a liquid carrier medium; and
- (ii) the subsequent extraction, with another supercritical fluid (SCF-E), after the dispersion of the substance liquid, of the liquid carrier medium.

10

2. A method according to claim 1, wherein the dispersion takes place in a nozzle spraying the dispersed mixture in a reaction vessel, the pressure and temperature of which is controlled leading to a supercritical state in the reaction vessel.

15

3. A method according to claims 1-2, wherein the extraction takes place inside the reaction vessel.

20

4. A method according to claims 1-3, wherein the first supercritical fluid has the lowest possible extraction capacity for the carrier medium and wherein the other supercritical fluid has the highest possible extraction capacity for the carrier medium.

25

5. A method according to any of the preceding claims, wherein the supercritical state is maintained inside the reaction vessel during the whole method.

6. A method according to any of the preceding claims, wherein the first supercritical fluid (SCF-D) has lower critical parameters than the second supercritical fluid (SCF-E).

7. A method according to any of the preceding claims, wherein the first supercritical fluid (SCF-D) is N_2 and the second supercritical fluid (SCF-E) is CO_2 .
- 5 8. A method according to any of the preceding claims, wherein the substance(s), of which the particles are prepared, are amorphous, semi-crystalline or crystalline.
9. A method according to any of the preceding claims, wherein the substance(s) is/are synthetical or natural polymers, solid organic substances, solid inorganic substances, salts, biomolecules such as proteins e g enzymes.
- 10 10. A method according to any of the preceding claims, wherein the liquid carrier medium is a solvent or a dispersing agent, or mixtures of one or more solvents or dispersing agents.
- 15 11. A method according to any of the preceding claims, wherein the carrier medium is chosen from water, lower ketones such as acetone, lower hydrocarbons such as hexane, lower alcohols such as methanol, ethanol, isopropanol, ethyl acetate, or mixtures thereof.
- 20 12. An apparatus for the preparation of particles, comprising:
- a particle formation reactor (2), the pressure and temperature of which are controllable;
- 25 a means (18, 2) in order to deliver to the apparatus a substance liquid comprising
- a) at least one substance of which the particles are to be prepared, and
- b) a liquid carrier medium for the substance;
- 30 a dispersing means (17) for the dispersion of the substance liquid and for the feeding of the dispersed substance liquid to the particle formation reactor (2);

a first feeding means (22, 17) for the feeding of a first supercritical fluid (SCF-D), with a minimal extraction capacity for the liquid carrier medium, to the dispersing means; and

5

a second, separately located, feeding means (12, 6) for the delivery into the particle formation reactor (2) of a second supercritical fluid (SCF-E) with a maximal extraction capacity for the liquid carrier medium, wherein the second supercritical liquid is introduced in the particle formation reactor (2) through a separate introduction channel.

10

13. An apparatus according to claim 12, wherein the dispersing means comprises a nozzle (17) with separate delivery channels for the substance liquid and the first supercritical fluid (SCF-D), respectively.

15

14. An apparatus according to claim 13, wherein the delivery channels are designed as a coaxial spray nozzle.

15. An apparatus according to any of the claims 12-14, comprising a first outlet channel placed in the upper part of the reaction vessel and a second outlet channel placed in the lower part of the reaction vessel.

20

16. An apparatus according to claim 15, wherein the outlet channels are provided with filters (26, 28).

25

17. An apparatus according to any of the claims 12-16, wherein the reaction vessel is a high pressure chamber and is provided with a thermostat and a pressure regulator.

30

18. Particles prepared by the method according to any of the claims 1-11, comprising one or more pure or mixed substances.

19. Particles according to claim 18, wherein the particles comprise one or more substances incorporated in one or several other substances.
- 5 20. Particles according to claim 18 or 19, comprising substances having activity as pharmaceuticals.

1/8

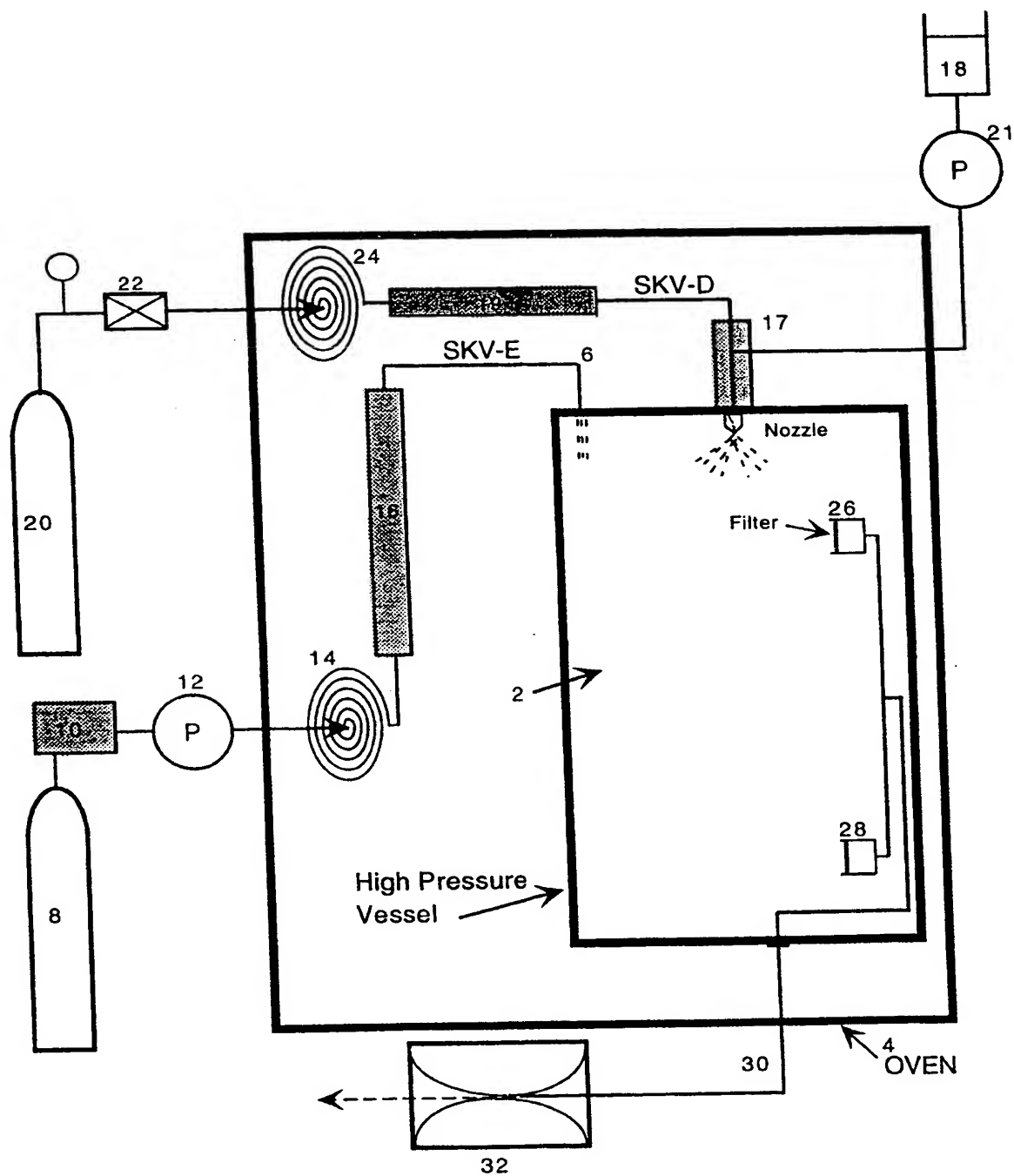


FIG. 1

2/8

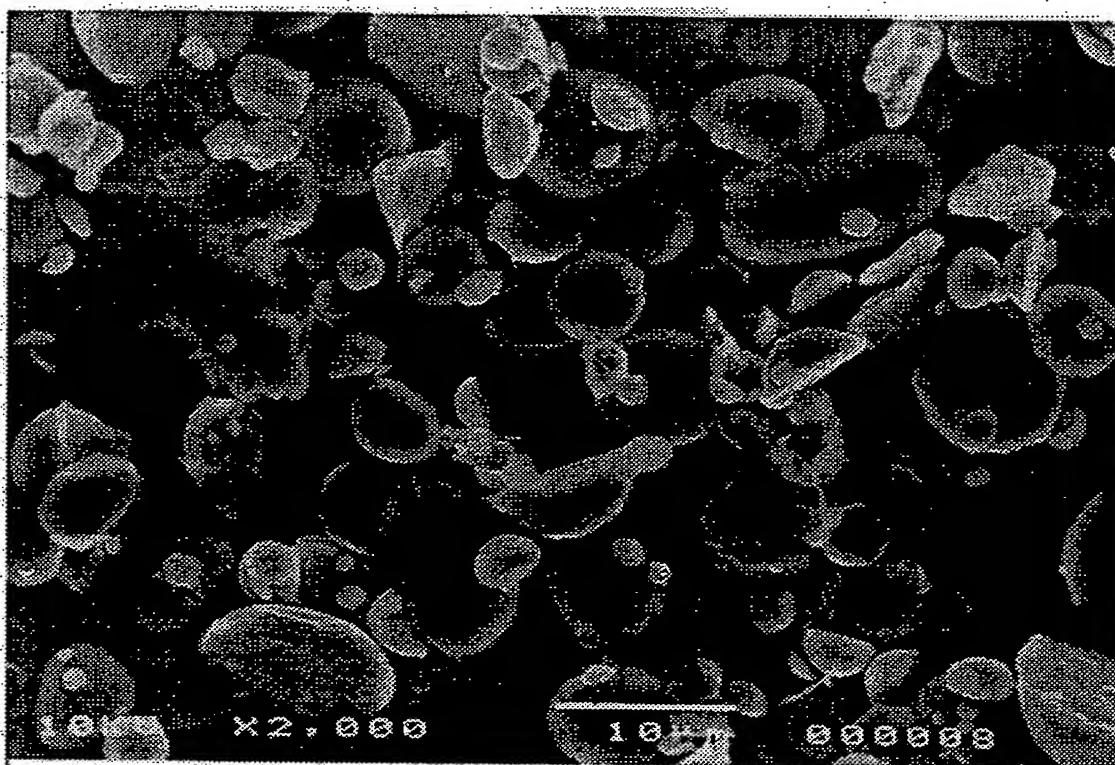


Fig. 2

3/8

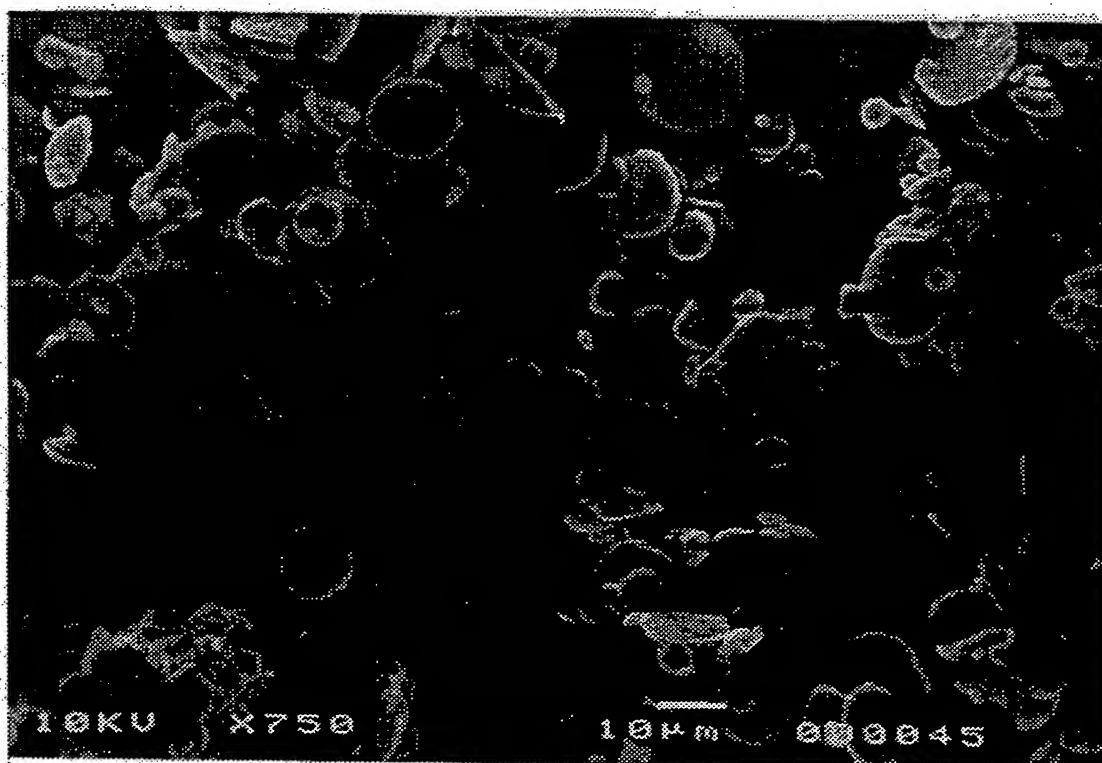


Fig. 3

4/8

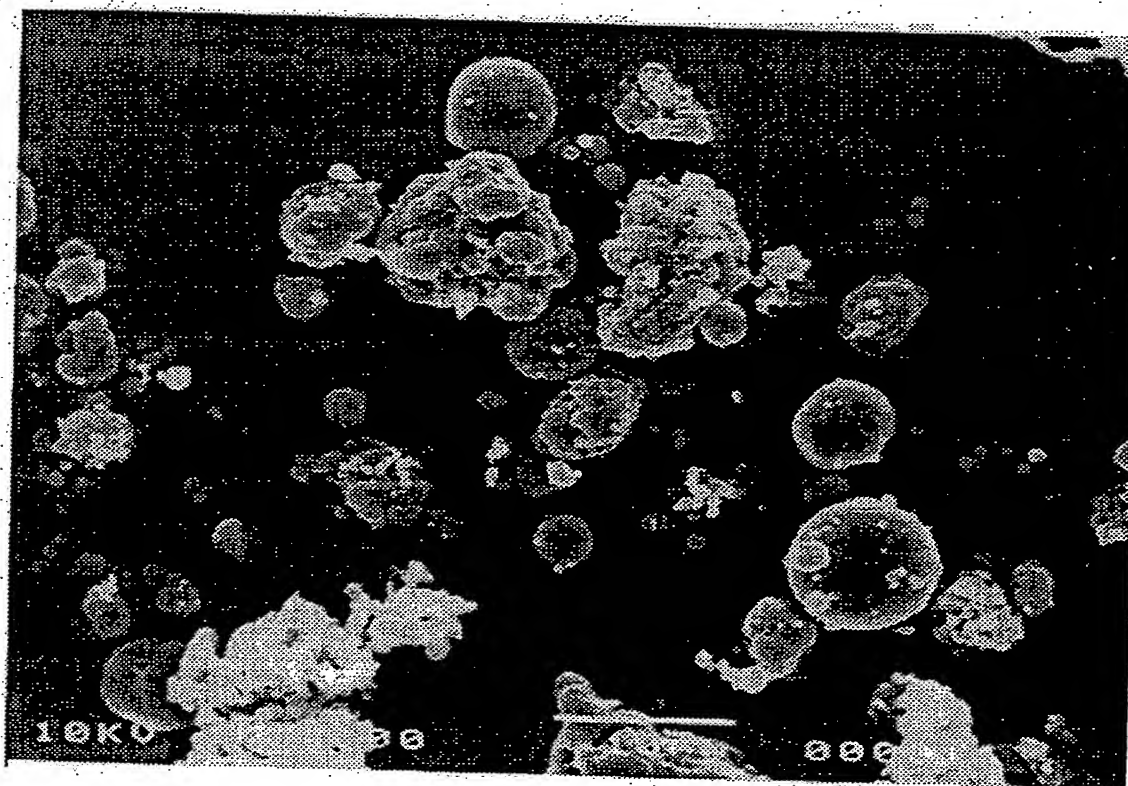


Fig. 4

5/8

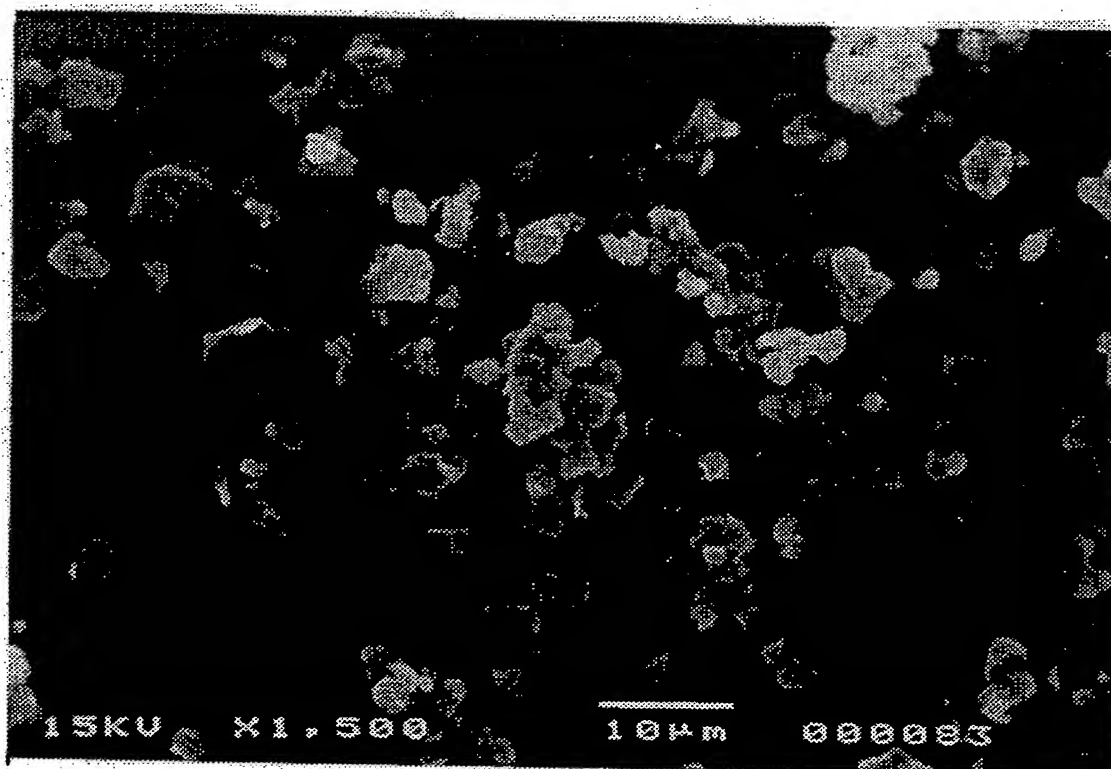


Fig. 5

6/8

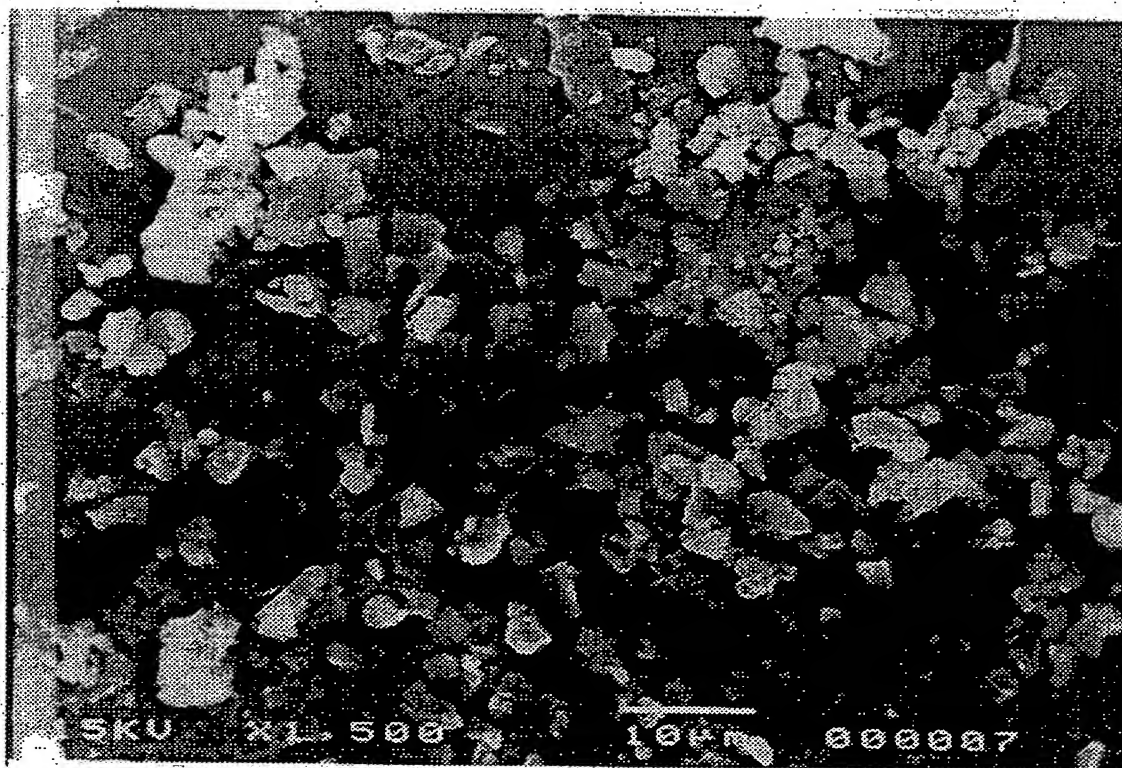


Fig. 6

7/8

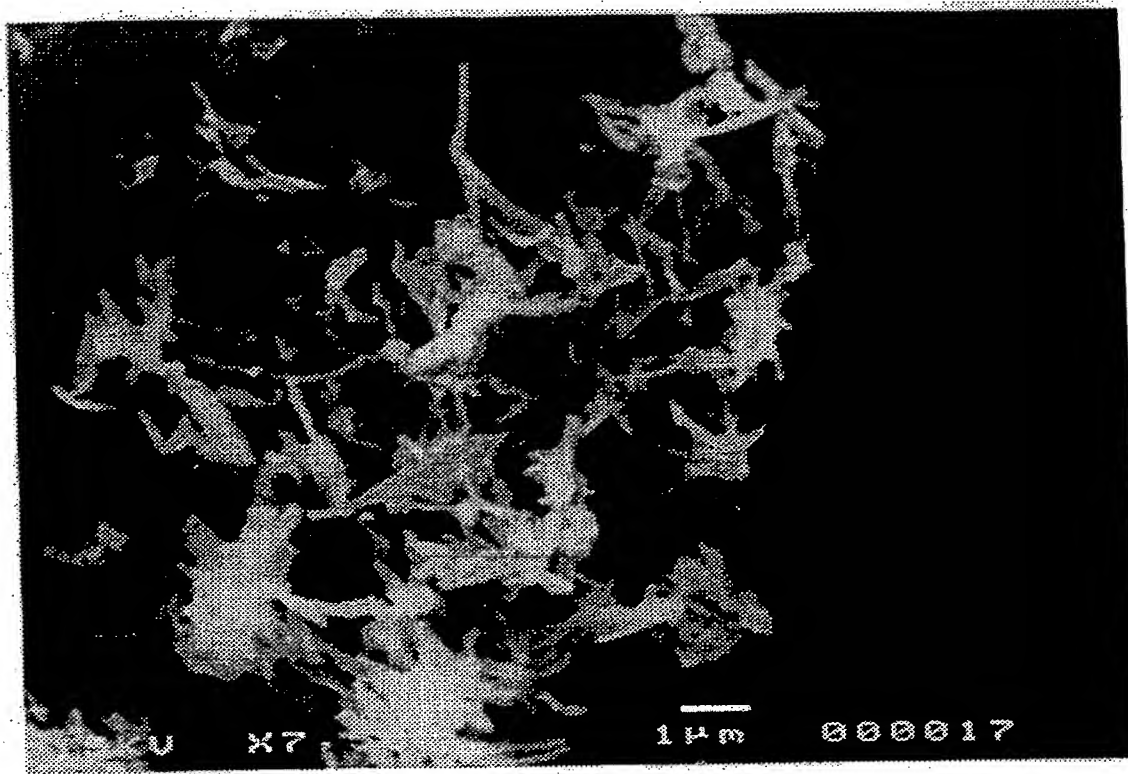


Fig. 7

8/8

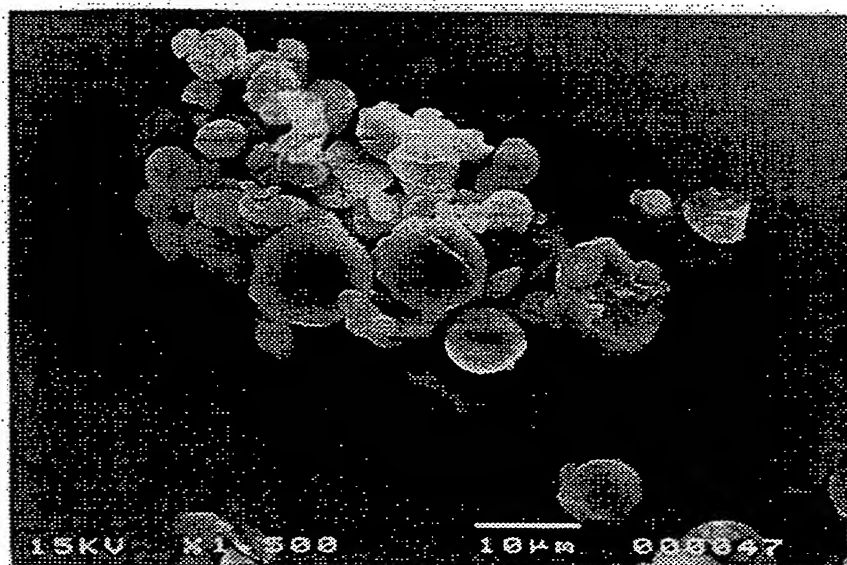


Fig. 8

INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 00/00566

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: B01J 2/04, A61K 9/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: B01J, A61K, A61J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9836825 A1 (BRADFORD PARTICLE DESING LTD.), 27 August 1998 (27.08.98)	1-3,5,7-20
Y	--	4,6
Y	JP 7008202 A (LION CORP) 1995-01-13 (abstract) World Patents Index (online). London, U.K.: Derwent Publications, Ltd. (retrieved on 1999-11-23) Retrieved from: EPO WPI Database. DW199512, Accession No. 1995-084299; & JP 7008202 (LION CORP) 1995-05-31 (abstract). (online) (retrieved on 1999-11-23). Retrieved from: EPO PAJ Database	4,6
	--	



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

28 June 2000

Date of mailing of the international search report

19 -07- 2000

Name and mailing address of the ISA/
Swedish Patent Office
Box 5055, S-102 42 STOCKHOLM
Facsimile No. +46 8 666 02 86

Authorized officer

Mårten Hulthén/MP
Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 00/00566

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9600610 A1 (UNIVERSITY OF BRADFORD), 11 January 1996 (11.01.96), claim 30, abstract --	18-20
A	EP 0125690 A2 (UNION CAMP CORPORATION), 21 November 1984 (21.11.84), claim 2 -- -----	4,6-7

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/SE 00/00566

Patent document cited in search report			Publication date	Patent family member(s)		Publication date
WO	9836825	A1	27/08/98	AU	6299898 A	09/09/98
				GB	2322326 A	26/08/98
				GB	9703673 D	00/00/00
				GB	9803634 D	00/00/00
				ZA	9801432 A	27/08/98

WO	9600610	A1	11/01/96	AT	174531 T	15/01/99
				AU	688526 B	12/03/98
				AU	2800395 A	25/01/96
				CA	2192811 A	11/01/96
				DE	69506707 D,T	01/07/99
				EP	0767702 A,B	16/04/97
				SE	0767702 T3	
				ES	2128743 T	16/05/99
				GB	9413202 D	00/00/00
				GR	3029531 T	30/06/99
				JP	10502016 T	24/02/98
				ZA	9505437 A	16/02/96

EP	0125690	A2	21/11/84	FI	841940 A	17/11/84
				US	4568495 A	04/02/86

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ **BLACK BORDERS**
- ☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- ☐ **FADED TEXT OR DRAWING**
- ☒ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- ☐ **SKEWED/SLANTED IMAGES**
- ☒ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- ☐ **GRAY SCALE DOCUMENTS**
- ☐ **LINES OR MARKS ON ORIGINAL DOCUMENT**
- ☐ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- ☐ **OTHER:** _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.

THIS PAGE BLANK (USPTO)